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Genetic predisposition to papillary thyroid cancer

Badanie predyspozycji genetycznej do raka brodawkowatego tarczycy

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Abstract

Approximately 5% of differentiated thyroid cancers are hereditary. Hereditary non-medullary thyroid cancer may occur as a minor component of familial cancer syndromes (e.g. familial adenomatous polyposis) or as a primary feature (familial non-medullary thyroid cancer [FNMTC]). Among FNMTC, PTC is the most common. Although a hereditary predisposition to non-medullary thyroid cancer is well established, the susceptibility genes are poorly known. Up to now, by linkage analysis using microsatellite markers, several putative loci have been described — 1q21, 6q22, 8p23.1-p22, and 8q24; however, validation studies have been unsuccessful. In the present review we discuss the results of linkage analysis and the most recent results of genome wide association studies (GWAS) with high resolution SNP (single nucleotide polymorphism) arrays. **(Pol J Endocrinol 2010; 61 (5): 486–489)**

Key words: *papillary thyroid carcinoma, familial non-medullary thyroid cancer, genetic predisposition, SNP*

Streszczenie

Około 5% zróżnicowanych raków tarczycy wykazuje predyspozycję dziedziczną. Dziedziczny nierdzeniasty rak tarczycy może występować jako składowa niektórych dziedzicznych zespołów nowotworowych, na przykład rodzinnej polipowatości jelit oraz jako rodzinny zróżnicowany rak tarczycy (FNMTC, *familial non-medullary thyroid cancer*), gdzie najczęściej obserwuje się raka brodawkowatego. Choć predyspozycja dziedziczna do nierdzeniastych raków tarczycy jest dobrze znana, to jednak geny warunkujące jej występowanie nie zostały jeszcze poznane. Wykonane jak dotąd badania zidentyfikowały kilka loci — 1q21, 6q22, 8p23.1-p22 oraz 8q24, jednak wyniki te nie zawsze były jednoznaczne. W niniejszej pracy omówiono rezultaty badań sprzężenia oraz ostatnio uzyskane wyniki badań związku całego genomu (GWAS, *genome wide association study*) wykonano przez badania polimorfizmu pojedynczego nukleotydu (SNP, *single nucleotide polymorphism*) z wykorzystaniem techniki mikromacierzy wysokiej gęstości. **(Endokrynol Pol 2010; 61 (5): 486–489)**

Słowa kluczowe: *rak brodawkowaty tarczycy, rodzinny zróżnicowany rak tarczycy, predyspozycja genetyczne, SNP*

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Introduction

Papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), poorly differentiated (insular) thyroid carcinoma (PDTC), and undifferentiated (anaplastic) thyroid carcinoma are all non-medullary thyroid cancer histotypes (NMTC) which originate from thyroid epithelial cells [1]. Among them, 5% constitute hereditary cases (HNMTC). Very rarely NMTC may occur as a component of familial cancer syndromes: Cowden's disease, familial adenomatous polyposis (FAP), Gardner's syndrome, Carney's complex type 1, Werner's syndrome, and papillary renal neoplasia, or as a familial non-medullary thyroid cancer (FNMTC) [2]. FNMTC is characterized by the presence of differentiated thyroid cancer of follicular cell origin in two or more first-degree relatives [2].

Among FNMTC, PTC is the most common; however, kindred with follicular and poorly differentiated thyroid cancer have also been reported [2]. The pathogenesis of papillary thyroid carcinoma (PTC) involves alterations in the RET/PTC-RAS-BRAF signalling pathway, most frequently by BRAF-activating somatic mutations and RET/PTC rearrangements [3, 4]. The loss of heterozygosity at the TCO and NMTC1 locus (loci linked with FNMTC) was demonstrated in some, but not all, tumour specimens from patients with FNMTC [2].

Genetic predisposition to PTC, which is the highest of all cancers not displaying Mendelian inheritance [5–7], is suggested by case-control studies, which show a 3- to

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10-fold higher risk in first degree relatives [3, 5, 6]. The risk is higher for first-degree male relatives of male probands than for first-degree female relatives of female probands [7]. Genes responsible for PTC /FNMTC are poorly known — genetic predisposition is expected to be multigenetic with low- to moderate-penetrance genes [4, 6] interacting with each other and with the environment determining individual susceptibility [6]. Up to the present, by linkage analysis using microsatellite markers, several putative loci have been described: TCO locus (thyroid tumours with cell oxyphilia) on 19p13.2 (first identified in a family with oxyphilic thyroid neoplasms), NMTC1 in 2q21 (identified in a family with hereditary transmission of the follicular variant of PTC), MNG1 (multinodular goiter) on 14q32 (a family with 18 cases of nontoxic multinodular goiter with 2 PTC individuals), and PRN1 locus on 1q21 (identified in a family with PTC, nodular benign thyroid disease, and papillary renal neoplasia) [2, 8–10]; however, in part, the results are contradictory. The reason for this are multiple: probably the imprecise FNMTC definition (two or more family members affected) and, as a consequence, dilution of the linkage studies with sporadic cases plays a role here. The heterogeneity of FNMTC syndrome also has to be considered [2].

The above-mentioned studies were performed on individual families, which were not necessarily representative of the vast majority of inherited cases, so validation of the results was unsuccessful [10]. Recently, new techniques — high resolution SNP arrays — have been used in genetic predisposition studies. Using this technique, genome-wide linkage analysis performed in a large family (11 members affected by nodular goiter and follicular thyroid adenoma, and 5 patients with thyroid cancer) revealed the linkage of region 8p23.1--p22 with familial thyroid epithelial neoplasia [11]. The same technique, used for linkage analysis performed on a large, broad sampling of 38 FNMTC families, discovered two distinct loci on chromosome 1q21, the first one described earlier as a PRN1 (papillary renal neoplasia) locus, and the second one previously unknown [10]. High density SNP arrays were also used to perform a genome-wide linkage study (large family with PTC and melanoma), which revealed the linkage of 8q24 locus with PTC. By using microsatellites markers the results were confirmed in 25 additional PTC families. Further analysis of the 8q24 locus implicated a putative non-coding RNA gene (AK023948) as a candidate gene for PTC [5].

In the absence of large pedigrees of related individuals, and to discover the chromosomal regions associated with common disease, SNP array-based techniques can be used to perform genome-wide association studies (GWAS) [10]. Association study seems to be an appropriate approach in multigenic disease as linkage analysis does not have sufficient power to identify low-penetrance genes [6]. Recently, a GWAS has been performed on 192 Icelandic patients with PTC or FTC and 37,196 healthy controls, in which 304,983 SNPs were tested for association [7]. The strongest signals were obtained for two SNP: rs965513 (A allele) on 9q22.33 and rs944289 (T allele) on 14q13.3. The results were confirmed in case control groups of European descent: 342 cases and 384 controls from the United States and 90 cases and 1343 controls from Spain. Combination of the results from Iceland, the United States, and Spain resulted in OR 1.75 for A allele of rs965513 ($p = 1.7 \times 10^{-27}$) and 1.3 for T allele of rs944289 ($p = 2.0 \times 10^{-9}$). In the general population, almost 11% of individuals were homozygous for rs965513 A allele, 32% for rs944289 T allele, and 3.7% were homozygous for both variants. Homozygous carriers of rs965513 A allele had a 3.1-fold higher risk for thyroid cancer then non-carriers, and a 1.9-fold higher for rs944289 T allele, respectively. The risk for doubly homozygous individuals was 5.7-fold greater. For the combined data, the frequency of rs965513 A allele carriers was higher among patient diagnosed at a younger age (rs944289 had no effect) [7]. There were no differences observed between males and females for rs965513 or for rs944289. Subsequently, the effects of two SNPs were analyzed in the combined data in the main histotypes of thyroid cancer (~ 85% of the Spanish and Icelandic cases were PTC, ~ 12% were FTC, whereas all of the United States cases were PTC). For rs956613 A allele the OR for PTC was 1.8 ($p = 4.7 \times 10^{-23}$) and for FTC OR was 1.55 ($p = 0.016$), and for rs944289 T allele OR was 1.32 ($p = 2.0 \times 10^{-6}$) and 1.63 ($p = 0.007$), respectively. This demonstrated that two SNPs increased the risk of two main histotypes of thyroid cancer (the numbers of other histotype cases in the combined data were too small to be considered) [7].

The SNP rs965513 lies on 9q22.33 within the linkage disequilibrium (LD) region where XPA, FOXE1, C9orf156, and HEMGN genes are localized, and the FOXE1 (forkhead box E1, formerly TTF2, Thyroid Transcription Factor 2) is the closest gene. FOXE1 is a centre of the regulatory network of transcription factors, which initiate thyroid differentiation at the embryonic stage. It is also important for the maintenance of the thyroid differentiated state in adults as it is involved in regulating the transcription of thyroglobulin and thyroperoxidase (TPO) gene, which are pivotal for thyroid hormone synthesis [6, 7]. The expression of FOXE1 has been shown to be abnormal in thyroid tumours, and its mutations among other phenotypes cause human syndromes associated with thyroid agenesis.

The SNP rs944289 on 14q13.3 is localized in the LD region where no RefSeq genes are present and the clos-

est genes are BRMS1L, MBIP, SFTA3, and NKX2-1 (TTF1). The best candidate as a source of the association signal obtained for the 14q13.3 locus is NKX2-1, which has a prominent role in thyroid development. Its expression is altered in thyroid tumours [7]. As both FOXE1 and NKX2-1 genes are involved in the biology of the thyroid gland, in the next step the effects of rs965513 and rs944289 on the TSH, free T_3 and T_4 serum levels were assessed. The measurement was taken over a period of 11 years on the material from Icelanders not having thyroid cancer. Both rs966513 A and rs944289 T alleles were associated with a decreased TSH serum concentration ($p = 2.9 \times 10^{-14}$ and $p = 0.03$, respectively). rs965513 A allele was associated with an increase in T_3 levels and with a decrease in T_4 levels ($p = 0.003$ and $p = 6.1 \times 10^{-5}$, respectively). For rs944289, no effects on either T_3 or T_4 were observed [7]. These data showed the influence of at least the rs945513 in the 9q22.33 locus on the thyroid function.

An independent, recently performed candidate gene association study also revealed the association of FOXE1 with PTC [6]. The study comprised "tag SNP" (used to infer LD blocks according to the HapMap project) and putative functional SNP in genes involved in thyroid cell differentiation and proliferation, and in genes found to be differentially expressed in thyroid carcinoma (as described in public databases CGAP-SAGE). A total of 615 Spanish cases and 525 controls were genotyped for 768 SNPs localized in 97 genes. The strongest evidence of association with PTC was observed for SNP in an LD block spanning the entire FOXE1 gene. The results were validated in an independent Italian series of 482 cases and 532 controls. The strongest association was observed for rs1867277 A allele localized in the promoter sequence, which was a different SNP to that observed in the Icelandic GWAS study. The combined OR (per allele) for rs1867277 was 1.49 ($p = 5.9 \times 10–9$). The FOXE1 in this study was particularly associated with the classic PTC variant. Functional assays revealed the recruitment of USF1 and USF2 transcription factors by rs186277 A allele, while both alleles, G and A, formed a complex in which DREAM, CREB, and αCREM participated. Transient transfection study assays revealed that CREB, and strongly αCREM, activated FOXE1 promoter, while DREAM reduced αCREM dependent-transcriptional induction. USF factors also induced significant increases in FOXE1 transcription activity when rs1867277 A allele was present. Transcriptional activation of the FOXE1 gene by binding transcription factors αCREM and CREB was regulated by hormonal factors, particularly by TSH via cAMP. The authors concluded that in this way transcription factors could regulate FOXE1 expression in response to TSH in a physiological situation, but also indicated that FOXE1-specific studies were needed to understand its role in thyroid tumour development. FOXE1 gene belongs to the forkhead family of transcriptional factors, which has recently been identified as a molecular signature for epithelial to mesenchymal transition in human colon cancer.

The authors hypothesized that increased FOXE1 expression in thyroid carcinomas (which has been observed to parallel the differentiation process of thyroid carcinomas) could be related to a motile advantage of malignant thyroid cells, which would be enhanced by the presence of the rs1867277 A risk predisposing allele [6]. Until now, no data are available on the expression of FOXE1 in PTC.

As mentioned previously, PTC may be caused by the interaction of multiple genes, either protein-encoding genes or regulatory genes. Recently, microRNA (miRNA) genes have been implicated in contributing to the pathogenesis of PTC. MicroRNAs are small noncoding RNA molecules which negatively regulate the expression of other genes. MicroRNAs are transcribed from endogenous DNA and they inactivate specific mRNAs and interfere with the translation of target proteins [4]. Thus, microRNAs regulate many processes like development, apoptosis, cell proliferation, and haematopoiesis; they also act as tumour suppressor genes and oncomirs. Expression of microRNAs has been found to vary between cancers and normal cells and among different types of cancers [4]. For example, miR-221, miR-222, and mir-146 are upregulated in PTC compared with unaffected thyroid tissue [3]. Recently it has been found that common G/C polymorphism (rs2910164) within the pre-miR-146a sequence is associated with PTC [4]. In material coming from 608 sporadic PTC patients and 901 controls from Finland, Poland, and the United States the frequency of genotype differed significantly ($p = 0.000002$). The G/C heterozygosity was associated with an increased risk of PTC (OR = 1.62; $p = 0.0000007$) in comparison with homozygosity, while both homozygous states were protective (OR = 0.42, $p = 0.0027$ for CC *v.* GG + GC; OR = 0.69, $p = 0.0006$ for GG *v.* CC + GC; OR = 0.5, $p = 0.024$ for CC *v.* GG). It was shown that this polymorphism reduced the amount of pre- and mature miR-146a from the C allele compared with the G allele. By evaluation of a combined group of DNA samples obtained from the blood of PTC patients and from normal thyroid tissue in patients with PTC, it has been found that 3.6–6.1% of PTC cases exhibit mutation from GG or CC in the germ-line DNA toward GC heterozygosity in the tumour. The authors concluded that polymorphism in pre-miR-146a might play a role in tumourigenesis. The effects of miRNA polymorphism were expected to be mediated by target genes whose expression was affected by the SNP status. miR-146a is known to be involved in NF-kappa

B regulation and the reduction in miR-146a leads to less efficient inhibition of target genes: RET-PTC1 (frequent rearranged proto-oncogene in PTC), TRAF6, and IRAK1 (involved in the Toll-like receptor and cytokine signalling pathway). The role of Toll-like receptors and NF-kappa B in thyroid tumourigenesis is well established [4]. It was also shown that miR-146a GC heterozygotes produced three mature microRNAs: one from the leading strand (miR-146a), and two from the passenger strand (miR-146a*G and miR-146a*C), each with its distinct set of target genes, whereas each homozygote produced two microRNAs (one from leading strand, the second from passenger strand). As shown by microarray and TaqMan real time PCR data, the difference in expression of miR-146a and other transcript between the tumour and unaffected parts of the thyroid from GC and GG patients can be observed. These data suggested that altered microRNAs might be early factors playing a role in the tumourigenesis of PTC [12].

To sum up, although many studies have been performed, up to the present the genes predisposing to FNMTC still seem not to be sufficiently identified. FNMTC tends to be more aggressive than sporadic cancer, is characterized by early age at onset, is often bilateral, and may have mixed PTC and FTC features [13]. Many medical centres recommend more aggressive treatment of FNMTC cases [2, 8, 10]. Identification of the susceptibility genes would enable FNMTC screening, early diagnosis, and as a result prophylactic treatment and improved patient outcome [2]. However, because of multigenetic predisposition to FNMTC due to the existence of heterogenous groups of histological variants, the issue is still a challenge and multicenter analysis would be necessary to reach the final goal [2].

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